

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**



In re U.S. Patent Application of

**YOSHIBA et al.**

} Art Unit 1638

Application Number: 10/026,767

} Examiner Ashwin D. Mehta

Filed: December 27, 2001

For: **TRANSGENIC RICE PLANT AND ITS FAMILY WITH  
ENVIRONMENTAL STRESS RESISTANT BY PROLINE  
ACCUMULATION OF HIGH LEVEL AND ITS  
PRODUCTION**

Attorney Docket No. NITT.0051

Honorable Assistant Commissioner  
for Patents  
Washington, D.C. 20231

**DECLARATION OF ONE SKILLED IN THE ART**

**UNDER 37 C.F.R. 51.132**

Sir:

I, Yosuke Yoshida, am a co-inventor of the invention claimed in the above identified application, and hereby declare as follows:

The transgenic rice plant of the invention introduced in sense orientation and tandemly connected to each other: (1) a P5CS gene of rice containing the sequence according to SEQ ID NO. 1, or a P5CS gene of *Arabidopsis thaliana* containing the sequence according to SEQ ID NO. 2, and (2) the antisense gene of a ProDH gene of *Arabidopsis thaliana*, said ProDH gene containing the sequence according to SEQ ID NO. 3.

Proline is one of the twenty amino acids that are used by living organisms as a building block of proteins, and it is regarded as playing multiple roles in environmental stress tolerance of plants. The present invention is directed to a rice plant able to accumulate a high level of proline. The transgenic rice plant according to the invention is introduced in sense orientation (1) a P5CS gene of rice (SEQ ID NO. 1) or *Arabidopsis thaliana* (SEQ ID NO. 2), and then (2) the antisense orientation of ProDH gene of *Arabidopsis thaliana* (SEQ ID NO. 3). For example in Fig. 1D, the introduction of a P5CS gene of rice (SEQ ID NO. 1) and then antisense orientation of ProDH gene of *Arabidopsis thaliana* (SEQ ID NO. 3) provide a synergistic effect of a significantly high level of proline of 1.15-4.62 mol/g FW.

On the other hand, the introduction of only one single gene (Fig. 2): a P5CS gene of rice (SEQ ID NO. 1), or a P5CS gene of *Arabidopsis thaliana* (SEQ ID NO. 2), or the antisense orientation of ProDH gene of *Arabidopsis thaliana* (SEQ ID NO. 3), only results in

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a proline concentration of 0.45-0.97 mol/g FW, 0.26-0.40 mol/g FW, or 0.27-0.78 mol/g FW, respectively, which is lower than that of the synergistic combination, but still higher than that of non-transgenic rices 0.07-0.18 mol/g FW.

Moreover, 95% of the transgenic rice with rice P5CS gene survived after 250 mM NaCl treatment for 72 hr. On the other hand, all non-transgenic plants were withered and dead after the treatment (Fig. 3).

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statement were made with the knowledge that willful false statements and the like so made are punishable by fine, or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the above-captioned application and any patent to issue thereon.

Respectfully submitted this 6 day of December, 2004

Yoshu Yoshioka  
Yoshu Yoshioka